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(54) **Automatic chemistry machine.**

(57) An automatic chemistry machine particularly suitable for the automatic sequencing of DNA on the microlitre scale includes a source of reagents, a common means to transfer by contact microlitre quantities of reagent, below 5 microlitres, and a rotatable reaction surface to which said quantities of reagent are transferable, means to control the transfer means to access and contact specific spaced areas of the reaction surface, and means to control the rotation of the surface with regard to the transfer means action, the surface being formed to constrain flow of transferred reagent quantities over the surface and in a reaction area to mix under centrifugal action on faster rotation of the surface.

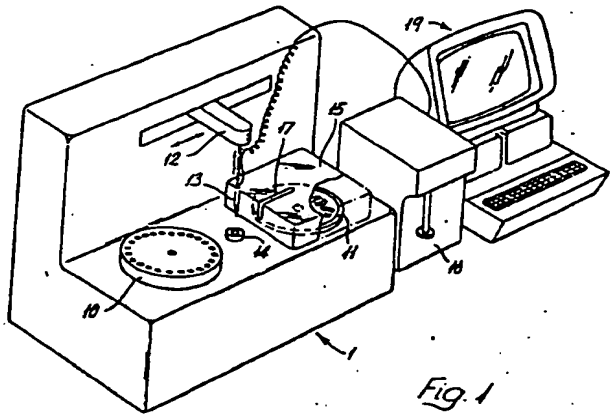

Fig. 1

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AUTOMATIC CHEMISTRY MACHINE

This invention relates to automatic chemistry machines.

Automatic chemistry machines have, in recent years, been the subject of considerable work in the field of analysis as the need to reduce the expenditure on skilled operators and the need to remove the variability and errors of such operators, however skilled, has led to attempts to carry out routine chemical procedures automatically. There is also a need to speed up procedures both to save operator time and make results available more quickly. Many mechanisms and techniques have been proposed including conveyors, turntables and shaking tables among others to convey vessels for reactions and suction, air bubbles and centrifugal action to move reagents through tubes. The clear conclusion from some twenty or more years of intensive work is that the automatic performance of any given chemical analysis procedure is not arrived at without a great deal of thought and experiment, usually requiring knowledge and experience of several scientific disciplines in one individual or group.

The technique for sequencing of DNA raises immense problems because of the sheer number of base-pairs involved and the need for absolute accuracy at every stage. The amount of information contained in a DNA molecule is enormous and it can take several years of work to determine the data contained in a molecule and enter it into a computer.

In DNA sequencing techniques microscopic quantities of reagent are handled in numerous tests to produce various DNA molecules. For economic reasons it is necessary to use microlitre quantities and the chemical procedure requires that measured quantities of various reagents are mixed in a particular order and with intermediate timed reaction intervals.

It is an object of the invention to provide an automatic chemistry machine particularly suitable for the chemical

techniques of DNA sequencing and also generally suitable for the measurement, deposition, mixing and reaction of microlitre volumes of reagents.

According to the invention there is provided an automatic chemistry machine including:

a rotatable reaction surface and means to support and rotate said surface,

means to deposit reagents on said surface by contact,

said surface including a smooth reaction area with alongside elongate radially extending flow constraining means to constrain said deposit reagents on rotation of the surface to flow freely over the reaction area in a radial direction and mix.

According to the invention there is also provided an automatic chemistry machine for the automatic sequencing of DNA on the microlitre scale including a source of reagents, a common means to transfer, by contact, microlitre quantities of reagent, below 5 microlitre, and a rotatable reaction surface to which said quantities of reagent are transferable, means to control the transfer means to access and contact specific spaced areas of the reaction surface, and means to control the rotation of the surface with regard to the action of the transfer means, the surface being formed to constrain flow of transferred reagent quantities over the surface and in a reaction area to mix under centrifugal action on faster rotation of the surface.

The reagent quantities may be between 1 and 5 microlitres for each reagent.

The surface may be a plate radially grooved or raised to receive reagent at spaced parts of the groove.

A microvolume reagent transfer device may be used to transfer reagent and/or deposit reagent in controlled volumes by contact with the surface.

According to one aspect of the invention there is provided an apparatus for carrying out chemical reactions under automatic control, the apparatus supporting side-by-side a rotary reagent store and a rotary reaction surface with a contact reagent transfer

means operable along a path radial over the surface to transfer selected reagents from the store for deposit on the reaction surface at selected respective positions by contact with the surface, the reaction surface being supported for rotation at a first, loading speed and at least a second higher, mixing speed, the reaction surface being formed to constrain the flow of reagents over the surface and in a reaction area to mix on rotation of the surface at a higher speed, the apparatus further including means to drive the reagent store, transfer means and reaction surface and control means settable and operable to co-ordinate the drive of the store, transfer means and surface to produce desired selective transfer of reagents to respective surface positions, the control means being further settable and operable to rotate the surface at a higher speed to mix the reagents and thereafter selectively transfer further reagent to the surface for further mixing.

According to the invention there is further provided a method of carrying out chemical reactions with microlitre quantities of reagents including providing a source of reagents, providing a reaction surface, individually transferring controlled microlitre quantities of reagents for a reaction from the source and depositing said controlled quantities spaced apart on the surface, and causing or permitting the deposited reagents to move together over the surface to bring about a desired reaction.

The reaction surface may be shaped so that movement in a specific manner causes deposited reagents to move in a controlled manner.

For example a disc of inert material may have at least one generally radial groove so that, on rotation of the disc, reagents deposited along the groove move under forces generated by the rotation of the disc to mix and react.

Conveniently if a sequence of reactions involving the addition of reagents at intervals is required earlier reagents may be deposited away from the centre of the disc and later reagents deposited inwardly of those already deposited so that contamination of any contact transfer means is avoided and any reaction does not start until rapid rotation of the disc moves the latest reagent.

A disc or other reaction surface may be prepared with reagents in advance of use and stored away from the machine in a suitable cool place to preserve the reagents.

The apparatus may include means to control the temperature of the reagents held in the reagent source. This may include means to supply heating or cooling or may be means to contain a cooling mixture such as aqueous urea or a dry ice/organic solvent mixture. Electrical or electronic heating and cooling may be used, e.g. the Peltier effect. The temperature of the reaction area and surface may also be controlled, generally or locally.

The transfer device may be controlled to move to different specific points on a radial path over the reaction surface to order the deposit of reagents.

Embodiments of the invention will now be described in which:-

Figure 1 is a schematic view of an automatic chemistry arrangement embodying the invention,

Figure 2 is a plan view of a reaction surface,

Figure 3 shows in partial plan views two further reaction surfaces,

Figure 4 is a cross-section of another reaction surface,

Figure 5 is a flow chart of a reaction which can be automatically carried out with the invention, and

Figure 6 is a flow chart of another such reaction.

Various machines for automatic chemistry are well-known. One such is GEMSAEC, widely described in the literature, devised about 1969 at Oak Ridge Laboratory under the direction of Norman Anderson for fast chemical analysis work using photometric observation of results. The essential feature of GEMSAEC was a turntable thick enough to house vertical or inclined wells for reagents and samples. The wells held up to 300 microlitre (0.3 millilitre) of reagent and as little as 1 microlitre of sample, for reaction with the reagent, was stated to be needed. The wells were separated by weirs so that the reagents and samples could not flow and mix. The wells were arranged in a radial line with a chamber for optical examination at the outer end of the

line. The chamber was linked by a tube formed by a bore through the turntable material to the nearest well. In operation the reagent of largest volume, in the innermost well of a line, could be forced over the weir to the next outer well by rotating the turntable rapidly enough. The reagent mixed with the sample and the mixture was displaced through the bore to the chamber. The bore ensured adequate mixing. Once in the chamber, the chemical reaction proceeded under observation as appropriate.

Figure 2 shows a reaction surface 2 embodying the present invention. A thin circular slab 20 of material such as PTFE is made suitable for rotation about its centre and the surface is formed into simple grooves such as 21 to provide reaction areas. A peripheral groove 25 is also provided. The slab is about 20 cm diameter and 1 cm thick. The grooves are smooth and regular in cross-section. As shown 24 grooves are in six groups of four. The grooves have a width of about 5 millimetres. More grooves can be used, e.g. 50 as 12 groups of four and two diametrically opposite blank grooves. The grooves, in the illustrated example, extend radially initially and then converge onto respective closely-spaced recesses 22 at the outer end of each groove, the recesses being in a straight line. This arrangement is not essential but is convenient as ganged four-probe liquid micro-probes are available readily and these can be used to extract the reacted chemicals. Other arrangements can be used but the inner part of the groove is preferably radial. The groove shape must prevent reagent or sample escape sideways when the speed of rotation changes. An important reason for the radial shape is that it eases the accurate deposition of reagent. The four dots indicated at 23 represent four discrete positions where reagents or samples can be deposited and remain where deposited for subsequent movement on rotation of the surface 2, about its centre, to then mingle and interact and end up in the respective recess 22. Clearly more positions can be used if needed. Positions nearer the centre can be used, when earlier deposited reagents or samples have been mixed by rotation, to avoid contamination. Any suitable microlitre delivery system

can be used but it is particularly convenient to use one in which an arm can be controlled to move along a radius of slab 20 by distinct increments to deposit microlitre quantities of respective reagents or samples at each of positions 23. This produces a common delivery system.

In use, reagents are deposited by contact between the probe and the disc surface at spaced positions, to avoid cross-contamination, and the slab 20 is then rotated at about 1700 r.p.m. for about half a minute to move the reagents to the recesses and mix them. The reagents are then left for about 15 minutes at ambient temperature (some 18°C) to allow the reaction to run to completion. If required, after a mixing rotation of the slab, further reagents can be deposited at intervals during a reaction period and moved to the recesses by a further short, fast, rotation of the slab. The material accumulated in recesses 22, about 10 microlitres in each case, is then removed with a suitable probe for further treatment, e.g. electrophoresis in a gel. Care must be taken to minimise evaporation of reagents by ensuring that they are not exposed for unduly long periods on the reaction surface. A close-fitting cover (not shown) may be fitted over the recesses to shield the reagents therein and to reduce evaporation. When radioactive reagents are used the cover is necessary to prevent spray escaping.

Figure 1 shows a complete apparatus embodying the invention for the automatic handling of reagents in DNA sequencing. Many of the mechanical and electrical details will be well-known and readily apparent to those skilled in the art and will not be described.

A case 1 houses two circular platforms 10, 11 which are supported for controlled motion about their respective axes. Platform 11 is essentially the item shown in Figure 2 and will be described later. Platform 10 is a reagent disc housing supplies of reagents in distinct tubes or cavities, which platform can be controllably moved by rotation to specific positions. Between the

two platforms is a support 12 for a microlitre probe 13. Support 12 is arranged to move probe 13 on the line of centres of platforms 10 and 11. Platform 11 has a cover 15 with a slot 17 for probe 13.

Platform 10 can be of any suitable form but is preferably arranged to provide a controlled temperature environment for the reagent supplies. Conveniently the latent heat of liquidification of a frozen material can be used to provide some degree of temperature control. The bags of material commercially available to cool picnic boxes are suitable and can maintain the platform 10 at or below $0^{\circ}C$ for several hours, without the need for cooling devices in the apparatus. Other cooling mixtures can also be used for specific sub-zero temperature ranges. The Peltier effect can also be used for heating or cooling. Heating and cooling can be selective on both platform 10 and platform 11.

Dilution or preliminary mixing of reagents can be carried out on platform 10 in preparation for deposition, the mixing and reaction implemented in platform 11. On platform 10 conventional non-contact deposition can be used, e.g. with the microlitre probe, to prevent cross-contamination of the probe.

Platforms 10 and 11 are operated by stepper motors to be indexable to bring any reagent tube or cavity of platform 10 and any radial groove of platform 11 on the line of action of probe 13 on support 12. In this way a chosen reagent from platform 10 can be placed at an appropriate point on platform 11 along a groove.

The manner in which platforms 10 and 11 and support 12 are moved and controlled will be readily apparent and not described further.

Platform 11 can also be rotated much more rapidly, as described with reference to Figure 2, to mix the reagents and bring about the desired chemical reaction.

The microlitre probe 13 is attached by a tube to a proprietary device shown at 16, such as the Microlab M (Hamilton) (RTM) having an accurate syringe to deliver volumes as small as 0.1 microlitre. A probe cleaning weir is shown at 14. The cleaning weir has one part supplied with clean water to flow over the weir into

a waste area and drain. The probe when dipped in the one part displaces water, to wash the probe and prevent cross-contamination. In operation the probe is supplied with a cleaning liquid. Some liquid is first expelled to clean the inside of the probe and the probe then dipped in the cleaning weir 14 to clean the outside and then withdrawn. An air bubble is then drawn into the probe tip to provide isolation from the cleaning liquid. The probe tip is then caused to move over and dip into a reagent in platform 10 and some reagent is collected by drawing back the liquid and air bubble in the probe. Some 10 microlitres is conveniently collected. The probe is then moved over platform 11 which is rotated as appropriate and the probe moved radially to the required position along a groove. Some 2 microlitres of reagent is then discharged from the probe by pushing the liquid and air bubble forward. This small quantity of reagent cannot form a drop and is transferred to platform 11 by touching the probe on the surface. Further small quantities of reagent can be transferred elsewhere on the platform. Other reagent quantities are possible, typically in the range 1 to 5 microlitres, but less or more may be needed in some applications. By a suitable sequence of movements, conveniently under the control of a microprocessor or the like, the platform 11 is supplied with the required disposition of reagents. Stepping of platform 11 is then changed to high speed rotation to bring about the mixing of the reagents and the reaction.

The recesses may taper and be curved to contain the mixed reagents and collect them for easy removal.

The exact speed profile can be adjusted to suit particular circumstances but in general a steady "ramp" of speed build up is desirable between the stepping speed and the mixing speeds. Too steep a ramp could lead to loss of reagent as spray or even displace the reagents sideways. If suitable controls are available an initially steep build up of speed followed by a slower approach to top speed is useful. Closed loop control would be advantageous.

Heating, or cooling, can be applied to the reagents in position on the reaction surface or when mixed to provide a specific temperature

for a chemical reaction. It can be useful to keep the surface spinning slowly during a reaction to assist mixing and to produce even heating.

Subsequently platform 11 can be removed for further processing of the reacted reagents and then cleaned for re-use. Alternatively a disposable platform can be used, e.g. a plastic moulding. Suitable locking and release arrangements will be apparent.

Deposition of reagent by always touching the probe on a clean part of the reaction surface ensures that the probe is not cross-contaminated and this reduces wash requirements.

The apparatus conveniently uses five stepper motors. One steps round and spins platform 11, another steps round platform 10. One moves support 12 and another drives the probe downwards and upwards from support 12. A fifth motor (in the Microlab M) measures and dispenses liquids from the probe. A suitable motor drive control system can readily be devised and appropriate instructions prepared and stored in a microcomputer 19 such as an Apple IIe or IBMPC (RTMs). The correct location of a reaction surface can be produced by keying it to the drive shaft and providing an indexing rotor fast on the drive shaft with a single radial slot in the rotor to identify an index position. When the motor stops after a high speed action it is then driven slowly until the index slot is detected by a sensor to cause the motor to stop with the reaction surface at a repeatable reaction surface position for the probe.

Figure 3 shows two other alternative versions of the rotatable reaction surface. In one version the reaction surface 30 of the slab 3 is formed to constrain the flow of reagent in reaction areas of straight radial grooves each of which, such as 31, has a small recess 32 at the outer end. The slab is a similar size to the one described above. No peripheral groove, such as 25 in Figure 2, is provided as this was to prevent water from a heating bath under the slab being spilled onto the reaction surface and contaminating the reagents. The straight grooves have a rectangular

cross-section and are about 3 millimetres wide and 2 to 3 millimetres deep. The sides are steep or vertical to prevent reagents in the groove escaping when the slab is rotated to mix the reagents.

It is possible to provide several separate grooves along a single radial direction, as outlined for the other version in Figure 3 where grooves 33, 34, 35 are in a single radial direction, separate with respective end recesses 36 from each other. In use it is possible to rotate the slab at specific speeds in a group so that at the slower speed first the outermost set of spacedly deposited reagents is mixed then at a higher speed the next inward set of reagents and so. Speeds such as 500, 900 and 1600 r.p.m form one possible group. Clearly the exact values will depend on the dimensions of the slab and groove position. It will be apparent that such a multiple groove system permits quite complicated operating regimes to be set up with one set of reactions being started a set time after another, for example, the reagents having been deposited in a single process. Reactions which require different incubation times can thus all be caused to be completed at one time. The exact regime is of course for the operator of the chemical process to decide from the facilities made available by the versatility of the apparatus. It may be convenient to load a further set of reagents while a previous mixture is reacting or incubating, as the stepping action can be useful to agitate the reacting mixture. The heating action should be confined to the recesses so three ring-like heaters can be used for this embodiment. The heating should not be applied so that reagent can evaporate before mixing.

The terminal recesses 22 are shown as being in groups of four in a straight line but, as mentioned above, this is only for one particular ganged probe and in Figure 3 the recesses, 32, 36, are positioned on a circle centred on the slab centre. The recess walls can be vertical or inclined.

Other forms of surface are possible which will constrain reagents to a reaction area on rotation of the surface. Radial raised portions such as walls, or ridges on the surface which

leads to recesses will constrain the flow of reagents when the surface accelerates so that the reagents flow into a recess at an outer end under centrifugal action and mix. If the direction of rotation is fixed only one wall, rib or ridge is required for each group of reagent deposits that are to mix.

The material of the reaction surface needs to be chosen with care. It is essential that there is no reaction between the surface material and the reagents, some of which are chemically very active. Polytetraflorethylene is a suitable material although not particular easy to work or cheap to buy. Dimensional stability could be a problem. Polypropylene is another material which is useful as it can be worked accurately and does not creep. A silicone coating may be used to resist chemical action and in this way less inert materials may be used for the body of the reaction surface. Disposable surfaces may also be used, again with a coating if needed. If the reaction surface is to be heated or cooled then suitable techniques may be applied for the construction of the surface.

Another form of washing arrangement for the transfer probe is possible. By placing the probe in a conforming cup and squirting cleaning liquid from the probe into the cup the outer side of the probe is washed by liquid swirling in the cup. When radioactive reagents are involved three washes are needed and all waste liquids must be disposed of properly.

In another version platform 11 is changed to four or more plates, one shown at 4, pivoted on a frame (not shown) to swing when the frame is rotated about the axis of the frame somewhat in the manner of a centrifuge. Each plate, which is conveniently square or rectangular, has an array of reaction areas (40) each having conical surfaces 41 leading to a recess 42. Figure 4 shows a cross-section through one row in the array.. The conical surface is capable of receiving spaced microlitre quantities of reagent, say four around the cone, and a fifth reagent may be placed in the recess. On rotation of the frame the pivoted plates swing to an angle at which the four reagents move into the recess to react with each other and any fifth reagent if present.

Typically each reaction area is a cavity 43 some 9 mm in diameter leading to the conical surface 41 which reduces the cavity to the recess 42 which is some 3 mm in diameter and about 3 mm deep, 1 mm of which depth is formed by a closed conical surface 44. The overall depth is some 8 mm. The areas are closely spaced (some 12 mm centres) in a block of about 15 mm thickness and 128 mm by 85 mm.

In both versions the microlitre quantities cannot move over the surface until subject to large forces on rapid rotation so no definite containers are needed nor any special mixing bores.

Figure 5 is a chart of a particular chemistry that the above apparatus is particularly suitable for.

The general form of the Sanger Chain Terminator reaction shown in Figure 5 is well-known. However a variant is shown in Figure 6 which is particularly appropriate for a machine such as is described above. Reaction surface discs can be prepared with primers automatically deposited in specific areas and then stored at $-20^{\circ}C$ for 3 months. The template can then be added and the primer/template annealed. In this form the disc will keep for 2 days at zero degrees centrigrade. For use the disc with annealed primer/template has an appropriate combined dideoxy/zero mix automatically deposited and a radioactive label added with the "Klenow" fragment. These and further steps are all automatic, up to the addition of the dye and the mix and spin action. The 15 minute period at $80^{\circ}C$ is manually controlled as is the loading onto gel for electrophoresis. However either or both of these steps can be automated.

To carry out the Figure 6 reaction on the reaction surface with multiple rings of areas superimposed deposition of some reagents can be used. Separate spaced deposition is used as far as the Klenow and radioactive label step. The primer/template and the zero mixes are deposited in outer positions with the Klenow in the innermost positions. These initial reagents are spun mixed and incubated then the dATP is deposited where the Klenow was initially deposited and is spun mixed and incubated in turn to so

that the dye can be deposited in its place for the next mixing step. Cross-contamination does not occur if the deposition probe touches a reagent deposition area where that reagent, such as dATP or Klenow, has been added to all the mixing cavities. Clearly such an area must be an inward one so that other reagents flow through them. Superimposed deposition permits the use of shorter reaction areas.

In particular the use of such small quantities reduces the ability of the deposited reagent to flow and in general ensures that no flow or mixing can occur until the surface is spun. No special shaping of the surface to keep deposited reagents in place is needed, apart from that to prevent lateral movement on acceleration so the surface can be simpler and cheaper to make and, if resuable, easier to clean.

The techniques provided by the invention are also useful for other chemistries that require the mixing of small quantities of reagents, with or without intervening mixing periods, such as those employed in DNA restriction mapping.

It will be appreciated that the exact sequence of operations such as reagent deposit position and sequence, spin speeds and duration, directly heating and cooling actions can be selected to permit a specific chemical procedure to be carried out automatically. Among these are "T-tracking", primer template annealing and "cloning" reactions. When the automatic chemistry machine is connected to a microcomputer or the device various speciifc chemical procedures can be stored in the microcomputer and called up from a "menu" to control the machine as required. Reagent selection can also be controlled in this way.

Reaction surfaces can be prepared in advance with some or all reagents and stored in a suitably cool place such as a freezer or refrigerator until required. The reagents would be the microlitre quantities deposited at spaced positions on the reaction surface. In this way less common reagents can be kept available without the need for preparing and storing the reagents, which are often of short life, locally for each use. By using controlled heating of

the reaction surface chemical reactions can be triggered. For example a reaction starting at 37°C can be triggered by raising the reaction surface to 40°C and then stopping the supply of heat or sensing the temperature and controlling the supply of heat.

Reagent storage discs, i.e. platform 10, can be similarly prepared in advance and stored safely. Only the templates have to be added to use the disc. As the templates are specific to a particular reaction a specific group of storage compartments can be provided which are filled and fitted in the disc in the users laboratory. When used these can be removed and different templates fitted in their place so that the "common" reagents already in the disc can be drawn on to the best possible extent.

CLAIMS

1. An automatic chemistry machine including:

a rotatable reaction surface and means to support and rotate said surface,

means to deposit reagents on said surface by contact,

said surface including a smooth reaction area with alongside elongate radially extending flow constraining means to constrain said deposit reagents on rotation of the surface to flow freely over the reaction area in a radial direction and mix.

2. A machine according to Claim 1 in which the means in the surface to constrain the reagents is at least one raised part.

3. A machine according to Claim 2 in which the raised part of the surface is one of a ridge, rib and wall.

4. A machine according to Claim 1 in which the means in the surface to constrain the reagents is at least one groove.

5. A machine according to Claim 4 in which the groove is straight.

6. A machine according to Claim 4 in which there are groups of grooves.

7. A machine according to Claim 1 in which the surface is of disc form and the means in the surface to constrain the reagents are each in part at least radial of the disc.

8. A machine according to Claim 1 including in said surface at least one open recess to receive reagents constrained to flow over the surface.

9. A machine according to Claim 1 in which the means to deposit reagents deposits only controlled microlitre quantities by contact of a liquid-carrying probe with said surface.

10. A machine according to Claim 9 in which the surface is a disc with a plurality of said constraining means each in part at least radial of the disc and in which the means to deposit reagents is movable over the disc and to a supply of reagents and operable to access specific spaced parts of the surface to deposit reagents from said supply by contact to be constrained to flow in a reaction area on said rotation.

11. A machine according to Claim 10 in which the means to deposit reagents is movable radially over the disc to specific parts of a reaction area spaced along a radius and the disc is selectively rotatable to bring specific parts to be contacted by the means to deposit reagent.

12. An apparatus according to Claim 1 in which the means to rotate the reaction surface is effective to rotate the surface at a first reagent deposition speed and second, higher, reagent mixing speed.

13. An apparatus according to Claim 12 in which the reagent deposition speed is a stepping motion.

14. An apparatus to carry out chemical reactions under automatic control, the apparatus supporting side-by-side a rotary reagent store and a rotary reaction surface with a contact reagent transfer means operable along a path radial over the surface to transfer selected reagents from the store for deposit on the reaction surface at selected respective positions by contact with the surface, the reaction surface being supported for rotation at a first, loading speed and at least a second higher, mixing speed, the reaction surface being formed to constrain the flow of reagents over the surface and in a reaction area to mix on rotation of the surface at a higher speed, the apparatus further including means to drive the reagent store, transfer means and reaction surface and control means settable and operable to co-ordinate the drive of the store, transfer means and surface to produce desired selective transfer of reagents to respective surface positions, the control means being further settable and operable to rotate the surface at a higher speed to mix the reagents and thereafter selectively transfer further reagent to the surface for further mixing.

15. Apparatus according to Claim 14 in which the store includes a plurality of storage compartments and the surface includes a plurality of reaction areas.

16. Apparatus according to Claim 15 including means to selectively adjust the temperature of at least one of the storage compartments and surface areas by at least one heating and cooling means and in which said control means is operable to cause said selected adjustment by operation of said means.

17. A method of automatically carrying out a sequence of chemical reactions under specific conditions with specific reagents including operating an apparatus according to Claim 16 under said control means to deposit selected reagents on a reaction surface for said reactions, rotate the reaction surface to mix the reagents, adjust the temperature of the reagents and the reaction surface, hold the reaction surface for a reaction time and conditions and deposit further reagents and rotate and hold the reaction surface to meet the reaction conditions.

18. A reaction surface for an automatic chemistry machine prepared with deposited reagents in selected positions and cooled to a temperature to preserve said reagents for eventual use in a specific chemical reaction.

19. A reagent store for an automatic density machine prepared with stocks of reagents for specific reactions and cooled to a temperature to preserve said reagents for eventual use for such reactions.

20. An automatic chemistry machine for the automatic sequencing of DNA on the microlitre scale including a source of reagents, a common means to transfer, by contact, microlitre quantities of reagent, each below 5 microlitre, and a rotatable reaction surface to which said quantities of reagent are transferable, means to control the transfer means to access and contact specific spaced areas of the reaction surface, and means to control the rotation of the surface with regard to the transfer means action,the surface being formed to constrain flow of transferred reagent quantities over the surface and in a reaction area to mix under centrifugal action on faster rotation of the surface.

21. A machine according to Claim 20 in which the surface is a plate radially grooved to receive reagent at spaced parts of the groove.

22. A method of carrying out chemical reactions with microlitre quantities of reagents including providing a source of reagents, providing a reaction surface, individually transferring controlled microlitre quantities of reagents for a reaction from the source and depositing said controlled quantities spaced apart on the surface, and causing or permitting the deposited reagents to move together over the surface to bring about a desired reaction.

23. Any novel feature or features alone or in combination herein disclosed.

24. An automatic chemistry machine substantially as herein described with reference to the accompanying drawings.

25. A method of automatically carrying out a chemical reaction substantially as herein described with reference to the accompanying drawings.

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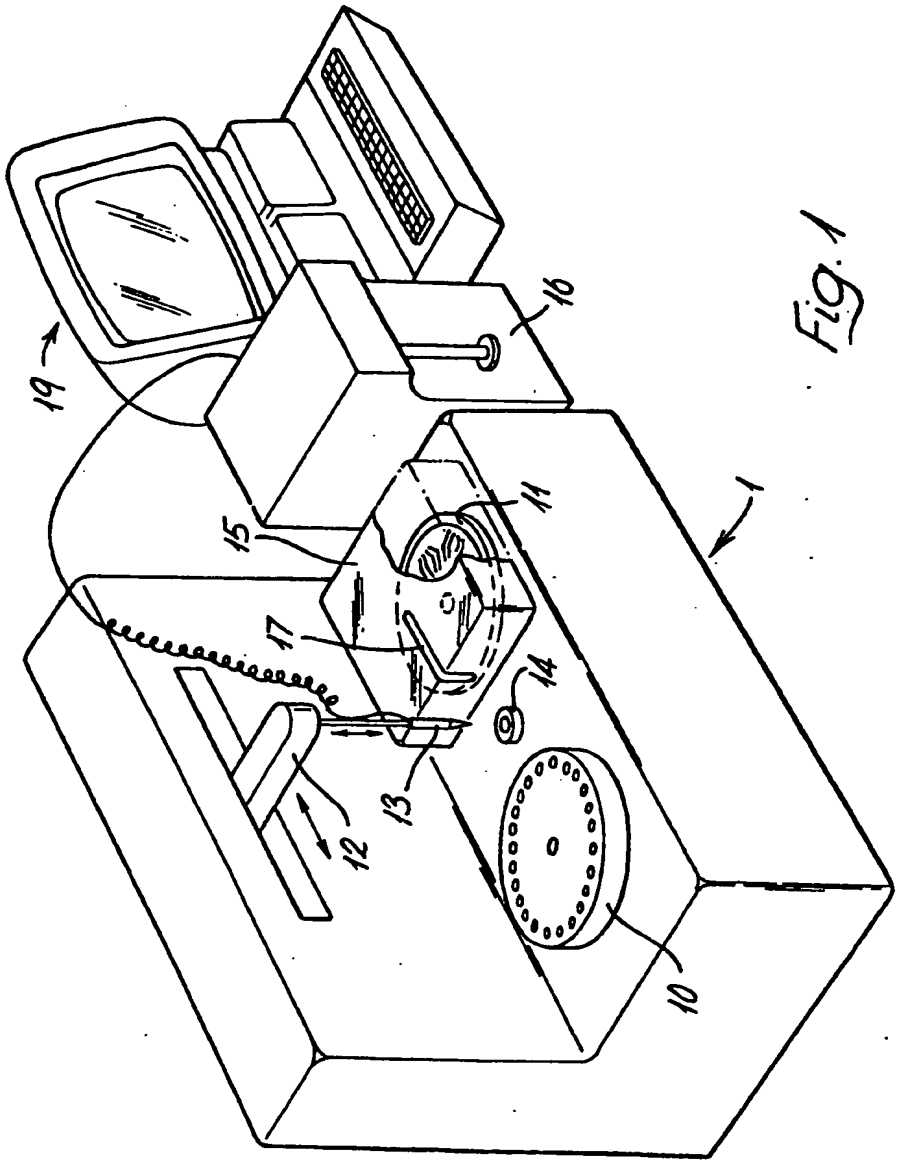

Fig. 1

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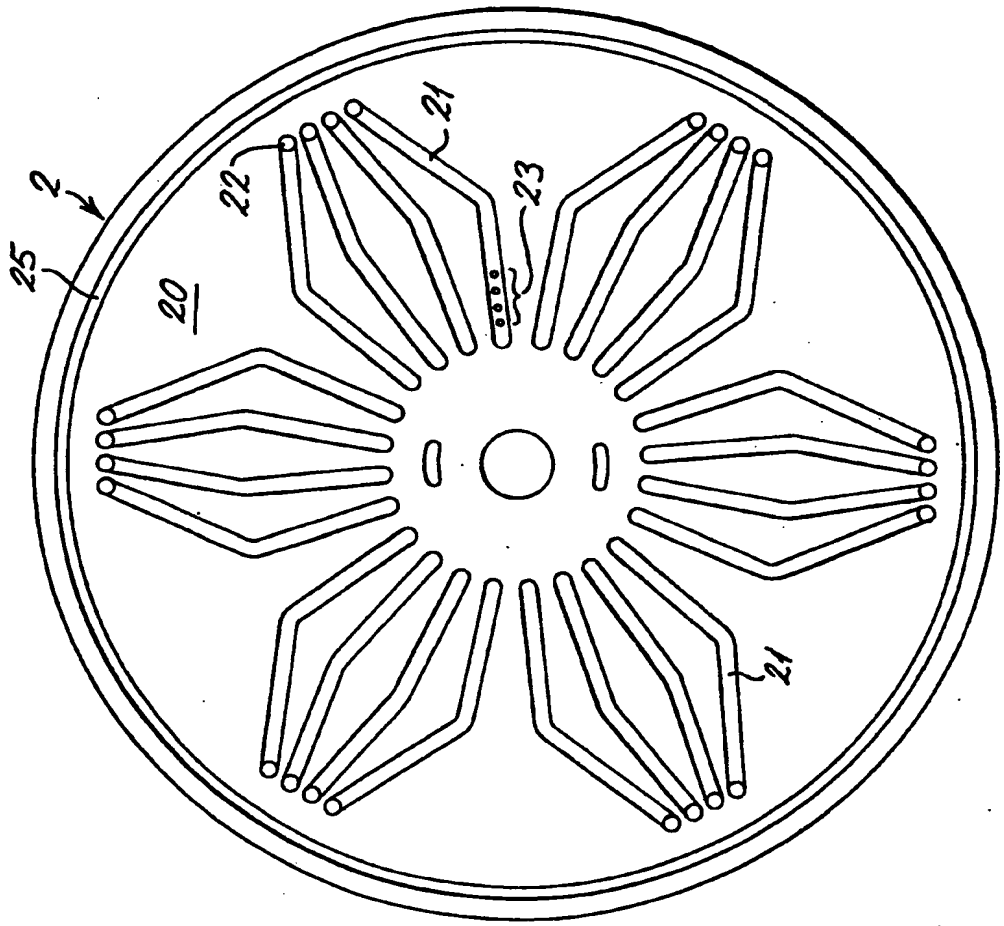

Fig. 2

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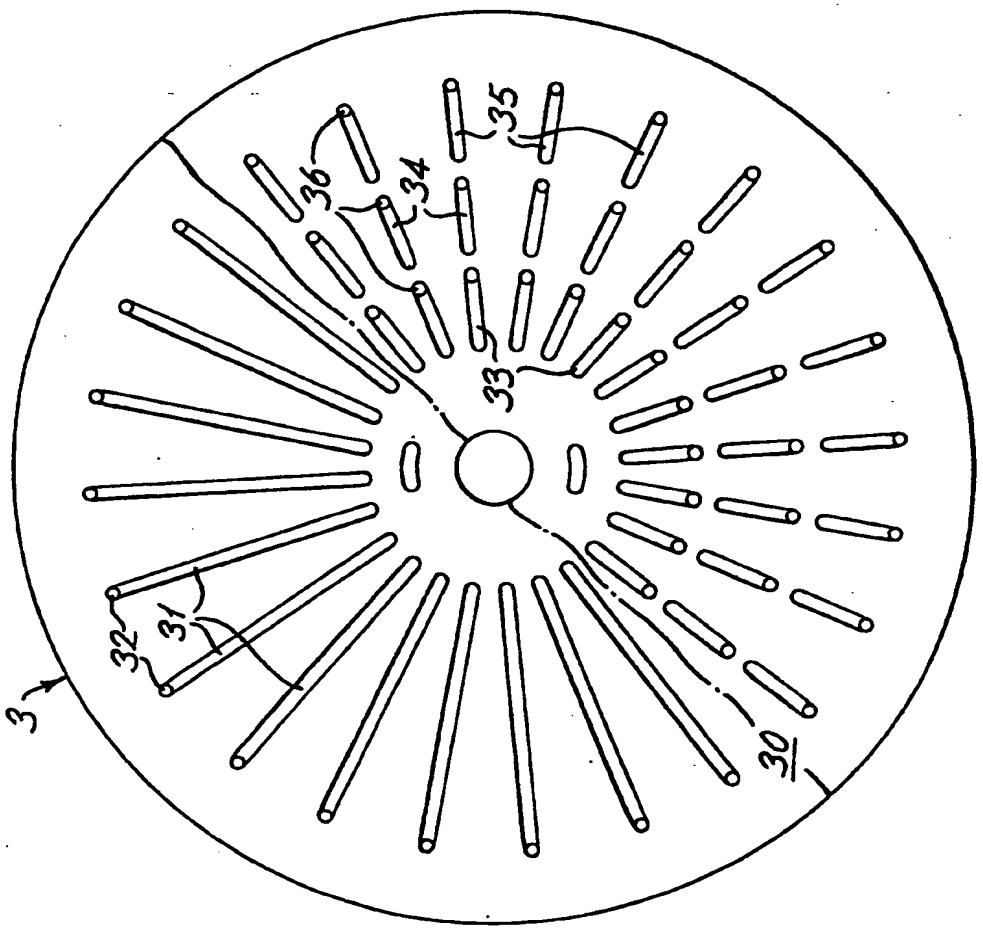
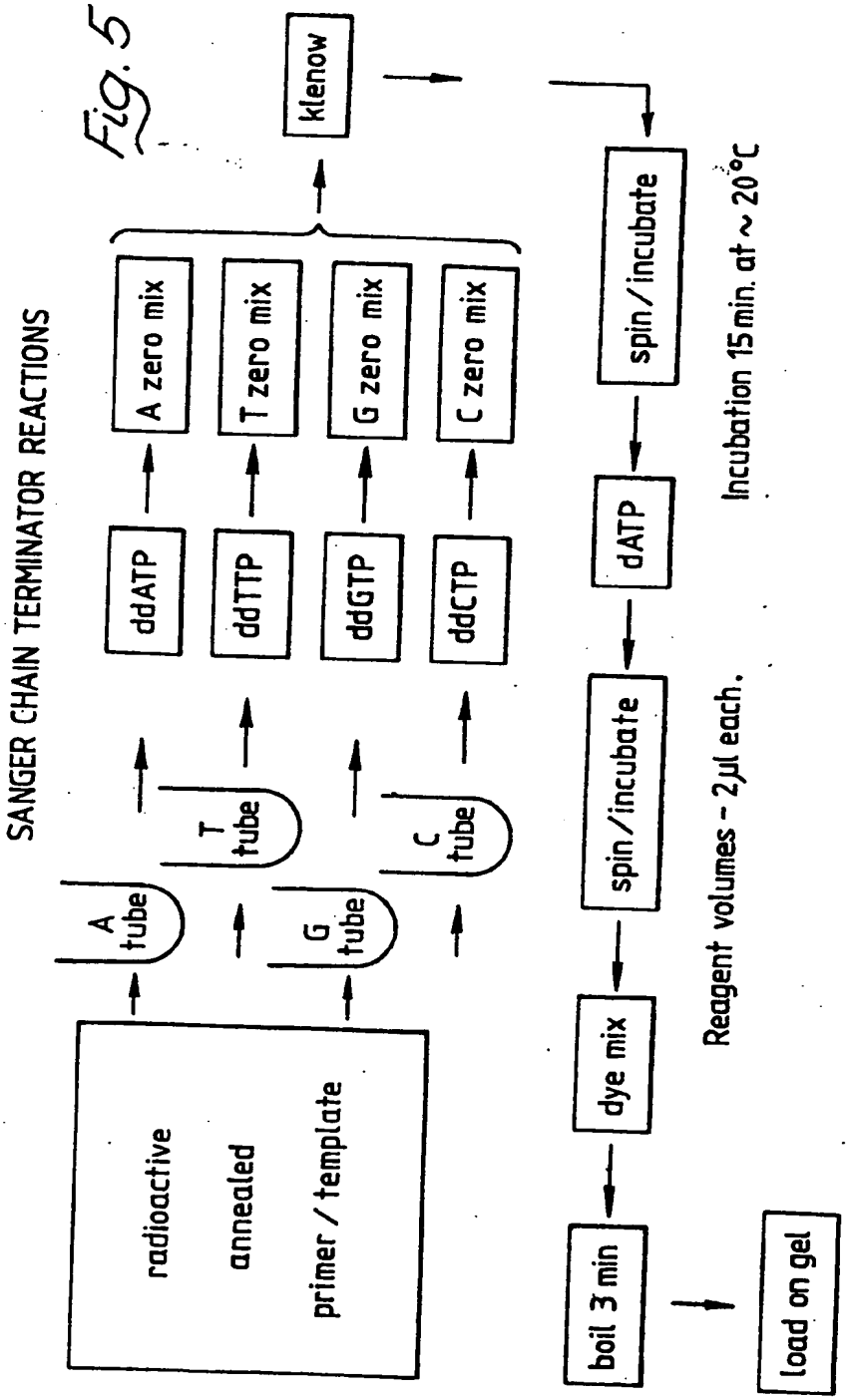


Fig. 3

Fig. 5


SANGER CHAIN TERMINATOR REACTIONS
radioactive
annealed
primer / template
A tube
T tube
G tube
C tube
ddATP
ddTTP
ddGTP
ddCTP
A zero mix
T zero mix
G zero mix
C zero mix
Klenow
spin / incubate
dATP
spin / incubate
dye mix
boil 3 min
load on gel
Reagent volumes - 2µl each.
Incubation 15 min. at ~ 20°C

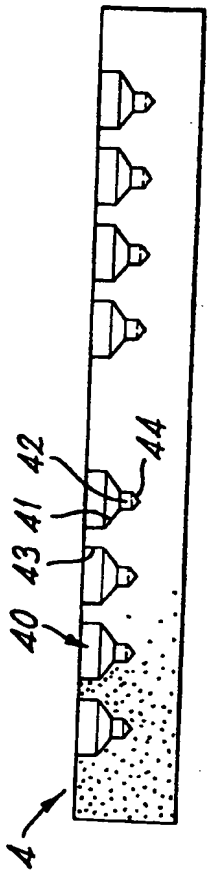

4
40
43
41
42
44

Fig. 4

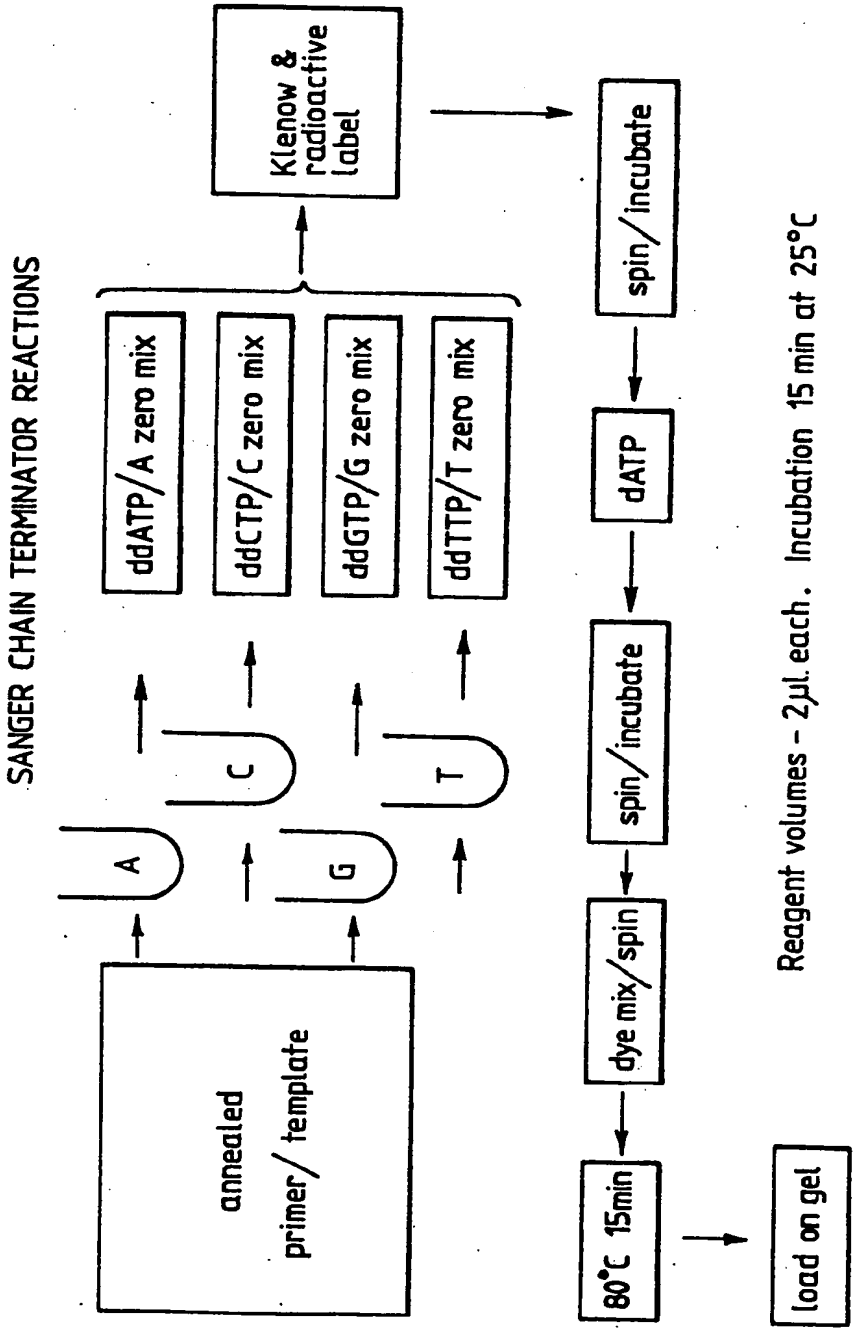

SANGER CHAIN TERMINATOR REACTIONS
annealed primer/ template
A
C
G
T
ddATP/A zero mix
ddCTP/C zero mix
ddGTP/G zero mix
ddTTP/T zero mix
Klenow & radioactive label
spin/incubate
dATP
spin/incubate
dye mix/spin
80°C 15min
load on gel
Reagent volumes - 2µl. each. Incubation 15 min at 25°C

Fig. 6